pH-Dependent Complexation of Poly(acrylic acid) Derivatives with Phospholipid Vesicle Membranes†

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ABSTRACT: Poly(acrylic acid), poly(methacrylic acid), and poly(α-ethylacrylic acid) can be used to modify, in a pH-dependent manner, the properties of phospholipid vesicle membranes. Polymer–lipid complexation causes a decrease in the apparent cooperativity of the lipid melting transition. The "critical pH" for complexation may be controlled through variation of the chemical structure and the tacticity of the poly(carboxylic acid). It is suggested that an important driving force for complexation is provided by the formation of hydrogen bonds between un-ionized carboxyl groups of the polymer and the phosphodiester functions of the lipid surface.

Introduction

Amphiphilic substances of appropriate molecular geometry form closed, spherical or ellipsoidal vesicles when dispersed in water with mechanical agitation or ultrasonic irradiation.[1] The vesicular membrane is a bilayer structure, and vesicles formed from natural or synthetic phospholipids are widely used as models for biological membranes. But phospholipids are not the only amphiphiles that form closed vesicles, and biomembrane modeling is not the only source of interest in such structures. Fendler, in particular, has cited the potential advantages of using vesicles in drug delivery, in solar energy conversion, and in chemical reactivity control,[2] and one might imagine additional applications in imaging, in sensing, or in medical diagnostics.

An important objective of current vesicle research is the preparation of vesicle membranes of enhanced stability with respect to chemical, osmotic, and mechanical stresses. The need for increased vesicle stability has motivated the preparation of polymerized phospholipid and surfactant vesicles in several laboratories.[3-10] This approach has been successful in a number of cases; Ringsdorf, for example, has produced polymerized vesicles that retain a nearly spherical shape even under the harsh dehydration and evacuation procedures required in the preparation of samples for electron microscopy.[4a]

A second objective of vesicle research is the design of membranes with properties that are sensitive to environmental parameters such as temperature[11] or pH.[12] One might imagine, for example, the use of such vesicles for selective release of drugs in targets of local hyperthermia[11] or low ambient pH.[12] Light-sensitive vesicles might release dyes in response to irradiation of a certain frequency or intensity, and chemically sensitive vesicles might be applied in sensing or diagnostic procedures.

We are exploring the possibility that synthetic polymers may be used to render vesicle membranes structurally stable and/or environmentally sensitive. Increased stability might be expected to result from a "polymerization" of the bilayer through association of the polymer with charged or polar sites of the amphiphile head group. Environmental sensitivity might be achieved by treatment of the vesicle suspension with a polymer that displays the desired sensitivity. In this paper, we report a pH-dependent complexation of several poly(acrylic acid) derivatives with phospholipid vesicle membranes, a complexation that renders the membrane properties sensitive to pH.[13]

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Experimental Section

Materials. Reagent grade toluene was refluxed over sodium for 24 h, distilled under N_2, and stored over sodium. Tetrahydrofuran (THF) was refluxed over and distilled from lithium aluminum hydride. *n*-Butyllithium (1.7 M in hexane), triisobutylaluminum (2.5 M in toluene), triethylaluminum (25% in toluene), and titanium isopropoxide were purchased from Aldrich Chemical Co. and were used without purification. Diisobutylaluminum diphenylamide was prepared by the reaction of triisobutylaluminum and diphenylamine in toluene at 60 °C and was used as the toluene solution. Azobis(isobutyronitrile) (AIBN) was recrystallized from methanol.

Methyl α-ethylacrylate was prepared as described previously[14] and distilled (bp 58 °C (50 mmHg)) over CaH_2 on a spinning-band column before use. α-Ethylacrylic acid was prepared by alkaline hydrolysis of ethyl α-ethylacrylate and fractionally distilled (bp 52 °C (1 mmHg)). Methyl methacrylate was freed of inhibitor and distilled from CaH_2. Poly(acrylic acid) (MW = 250 000) was purchased from Aldrich Chemical Co.

Dipalmitoylphosphatidylcholine (DPPC), dimyristoylphosphatidylcholine (DMPC), distearoylphosphatidylcholine (DSPC), and dipalmitoylphosphatidylglycerol (DPPG) were purchased from Sigma Chemical Co. and were used without purification. Thin-layer chromatography revealed no impurities in any of these lipid samples.

Tris(hydroxymethyl)aminomethane hydrochloride (Tris-HCl) was purchased from Sigma Chemical Co. as the preset pH crystals (pH 7.0, 7.4, 8.0, and 9.0). Buffer solutions of other pHs were prepared by adding concentrated HCl to solutions of preset crystals.

Synthesis of Poly(α-ethylacrylic acids) (PEAAs). Anionic Polymerization. A glass ampule equipped with a three-way stopcock was evacuated, dried with a hot-air gun, and then filled with dry N_2. Toluene (15 mL) and methyl α-ethylacrylate (2.8 g, 27 mmol) were added via syringe. The monomer solution was cooled, and a solution of *n*-butyllithium in toluene (1.27 mmol) was injected. The ampule was sealed under N_2 and placed in a cooling bath at the desired temperature (cf. Table II). Polymerization was stopped by adding a small amount of methanol, and the polymer was precipitated by pouring the reaction mixture into a large excess of methanol. After standing overnight, the polymer was collected by filtration, washed thoroughly in methanol, and dried in vacuo at 55 °C.

Poly(methyl α-ethylacrylate) was dissolved in concentrated H_2SO_4 to produce a solution containing 0.1 mol of repeating units per liter. The solution was stirred at room temperature for 10 days and poured into excess ice water. The precipitated polymer was collected by filtration and redissolved in 1 N KOH. After 3 h at 80 °C, the basic solution was added to dilute aqueous HCl to reprecipitate the polymer. The polymer was collected by filtration and dried in vacuo at 55 °C. The hydrolysis and isolation procedures were performed twice in order to ensure complete conversion. The basic hydrolysis is required in order to cleave the anhydride rings that form in H_2SO_4; the infrared bands at 1795 and 1750 cm^{-1} in the intermediate polymer disappear during the basic hydrolysis.

Radical Polymerization. Radical polymerization of α-ethylacrylic acid in bulk was run in a sealed tube under N_2, with 0.5 mol % AIBN as initiator (Table II). The polymer was dis-

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Table I
Preparation of Poly(methacrylic acids)

preparation of PMMA precursor					characterization of PMAA			
						triad tacticity, %		
initiator	solvent	temp, °C	time, h	yield, %	η_{inh},[a] dL/g	I	H	S
BuLi	THF	0	24	44	0.85	59	20	21
$AlEt_3/Ti(O\text{-}i\text{-}Pr)_4$	toluene	−78	24	83	2.05	1	2	97

[a] 0.2% in DMF, 35 °C.

Table II
Preparation of Poly(methyl α-ethylacrylates) of Variable Microstructure[a]

							triad tacticity, %		
expt	initiator	solvent	temp, °C	time, h	yield, %	η_{inh},[b] dL/g	I	H	S
1	BuLi	toluene	0	168	6	0.98	91	7	2
2	BuLi	toluene	−40	168	84	0.49[d]	77	16	7
3	BuLi	toluene	−78	120	68	0.66[e]	49	18	33
4	$(i\text{-Bu})_2AlNPh_2$	toluene	−40	168	11	6.2	1	11	88
5[c]	AIBN	none	60	48	41	0.34[f]	16	44	40

[a] By polymerization of methyl α-ethylacrylate, except experiment 5. [b] 0.2% in toluene, 30 °C. [c] By polymerization of α-ethylacrylic acid and subsequent methylation with CH_2N_2. [d] η_{inh} (0.2% in DMF, 35 °C) of PEAA after hydrolysis = 0.44 dL/g. [e] η_{inh} (0.2% in DMF, 35 °C) of PEAA after hydrolysis = 0.39 dL/g. [f] For PEAA before methylation.

solved in pyridine and precipitated in aqueous HCl. After standing overnight, the polymer was collected by filtration and dried in vacuo at 55 °C.

Synthesis of Poly(methacrylic acids). The procedures used for anionic polymerization of methyl methacrylate and hydrolysis of the resulting polymers were identical with those described above for methyl α-ethylacrylate. The characteristics of the poly(methacrylic acids) used in this work are summarized in Table I.

Measurements. ^1H NMR spectra were recorded on a Bruker WM-300 spectrometer with tetramethylsilane as internal standard. The following solvents and temperatures were used: for poly(methyl α-ethylacrylates), nitrobenzene-d_5, 150 °C; for poly(methyl methacrylates), $CDCl_3$, 58 °C; for poly(methacrylic acids), *N,N*-dimethylformamide-d_7, 100 °C.[15]

The tacticity of the poly(α-ethylacrylic acid) sample prepared by radical polymerization was determined after methylation of the polymer with diazomethane. *Caution*! Diazomethane is both explosive and toxic! Poly(α-ethylacrylic acid) was suspended in dioxane at a concentration of 5 mg/mL. A cold ethereal solution of diazomethane was added slowly, dropwise, until the yellow color of the solution no longer faded. The solution was then stirred for 12 h and poured into ether to precipitate the polymer. After filtration and drying, the tacticity of the polymer was determined as described above.

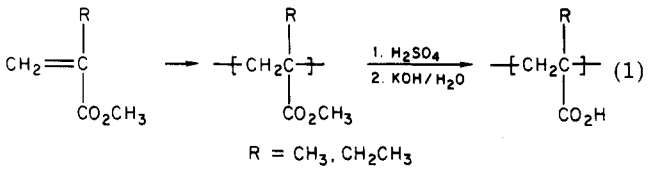
Calorimetric scans were recorded on a Microcal, Inc., MC-1 scanning microcalorimeter. A heating rate of 10 °C/h was used in all experiments. Lipid suspensions were prepared by vortex agitation of dry lipid (10 mg) with 50 mM Tris-HCl buffer (10 mL) which contained the polymer of interest (10 mg). All polymer solutions were prepared from doubly distilled, deionized water and dialyzed overnight against fresh buffer in order to remove low molecular weight contaminants. Cellulose dialysis tubing with a molecular weight cutoff of 1000 was used; the loss of polymer during dialysis was in all cases less than 7%. After vigorous agitation of the polymer–lipid mixture at a temperature above that of the lipid phase transition, the suspension was degassed and transferred to the calorimeter via calibrated syringe. Enthalpy measurements were run at least in triplicate and were calibrated by a precisely known current supplied to the reference cell of the calorimeter. Error limits on transition enthalpies are given as ±1 standard deviation.

Of the three thermal phase transitions that are known to occur in aqueous DPPC suspensions, we discuss only the main transition. In general, the behavior of the pretransition was found to be similar to that of the main transition. In no sample did we observe a subtransition, nor did we attempt to do so.

Solution pH was determined with a Fisher Accumet Model 325 pH meter. Because the pH of Tris-HCl solutions is temperature dependent, the pH at the lipid phase transition temperature differs somewhat from the pH of the suspension as prepared at room temperature. Unless otherwise noted, stated pHs are those determined at room temperature.

Results and Discussion

Polymer Synthesis. The synthesis of poly(methacrylic acids) of known tacticity has been accomplished by many previous workers, via polymerization of methyl methacrylate and subsequent hydrolysis. In this work, we adopted a similar scheme for the preparation of poly(α-ethylacrylic acids) of known microstructure (eq 1). Table II summarizes the results of these polymerizations.



The results of experiments 1–4 are in good agreement with those of Hatada et al.[16] In particular, we have confirmed their ^1H NMR method for determining the tacticities of poly(methyl α-ethylacrylates), and at the higher field available to us (300 MHz vs. 100 MHz in ref 16), we obtained the well-resolved spectra shown in Figure 1. The singlet at δ 2.2 in Figure 1A arises from the backbone methylene protons of syndiotactic dyads and allows the ester methyl signal at highest field (δ 3.69) to be assigned to syndiotactic triads. In similar fashion, the prominent AB quartet centered at δ 2.2 in Figure 1B requires that the downfield ester methyl line (δ 3.74) be assigned to isotactic triads. The triad tacticities listed in Table II were determined by integration of the ester methyl signals. The microstructures of the polymers obtained in experiments 1–4 are quite consistent with those found by Hatada et al. under similar conditions. The small differences that do arise may result from differences in polymerization temperature (which was not closely controlled in our work) or from differences in resolution of the NMR spectra.

Also listed in Table II is a radical polymerization of α-ethylacrylic acid, which gave the desired polyacid directly. Several attempts to determine the microstructure of the polyacid failed (including the use of DMF-d_7 at 100 °C, which works nicely for poly(methacrylic acid)[15]), so the polymer was esterified with CH_2N_2, and the NMR spectrum of the resulting poly(methyl α-ethylacrylate) was recorded. The triad tacticity listed in Table II indicates that poly(α-ethylacrylic acid) produced by radical polym-

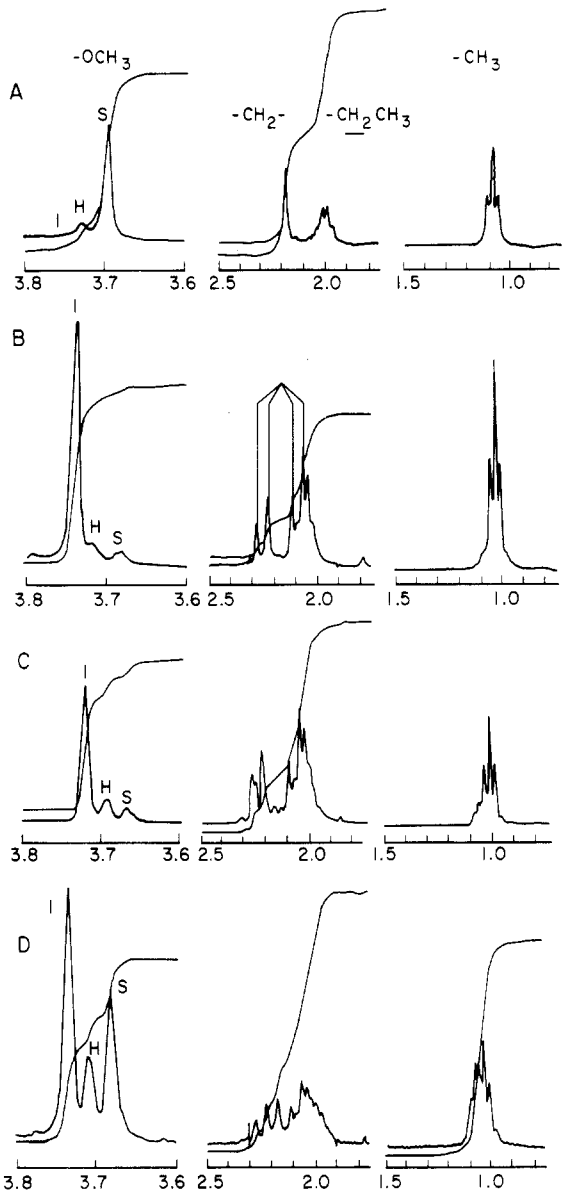


Figure 1. 300-MHz ^1H NMR spectra of poly(methyl α-ethylacrylates) prepared by anionic polymerization. Spectra were recorded at 150 °C in nitrobenzene-d_5. Polymerization conditions are given in Table II, and the following numbers of polymerization experiments refer to that table: (A) experiment 4; (B) experiment 1; (C) experiment 2; (D) experiment 3.

erization at 60 °C is stereoirregular, with a slight predominance of syndiotactic triads.

Hydrolysis of the poly(methyl methacrylates) and poly(methyl α-ethylacrylates) was accomplished by treatment with H_2SO_4, followed by aqueous KOH. The extent of hydrolysis was found to be at least 98%, as determined from the complete absence of the ester methyl signal from the 60-MHz ^1H NMR spectra of the polyacids.

Interaction of Poly(α-ethylacrylic acids) with Dipalmitoylphosphatidylcholine Vesicles. Effect of Tacticity. Because of our interest in pharmaceutical applications of synthetic polymers and polymer–lipid mixtures, we first investigated the interaction of poly(α-ethylacrylic acids) (PEAAs) with vesicles of dipalmitoylphosphatidylcholine (DPPC) at physiological pH (pH 7.4). The phosphatidylcholines are important constituents of the membranes of mammalian cells, and DPPC in particular forms comparatively stable vesicle suspensions in aqueous media. Hydration of DPPC with mechanical agitation in excess water produces a suspension of

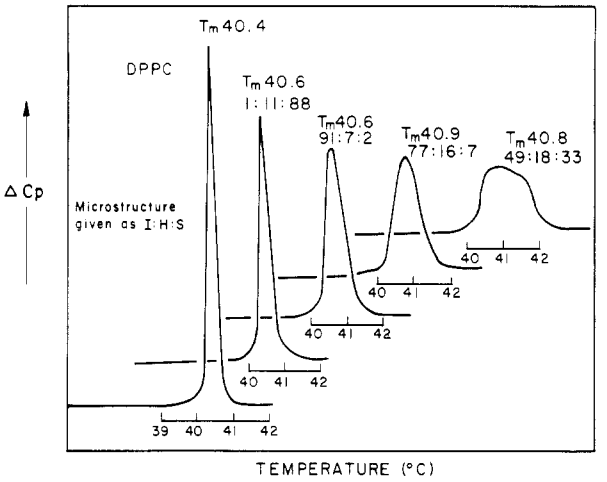


Figure 2. Main phase transition region for DPPC multilamellar vesicles (1 mg/mL) prepared in 50 mM Tris-HCl buffer (pH 7.4) that contained poly(α-ethylacrylic acid) at a concentration of 1 mg/mL. The leftmost endotherm is that of DPPC alone; the microstructure of the polymer added to each of the other suspensions is given near the peak of the melting endotherm for that suspension.

Table III
Modification of DPPC Phase Transitions by Poly(α-ethylacrylic acids): Effect of Tacticity[a]

polymer[b]	T_m,[c] °C	ΔH_m, kcal/mol	$\Delta T_{1/2}$,[d] °C
none	40.4	7.60 ± 0.35	0.21
1/11/88	40.6	7.85 ± 0.72	0.30
91/7/2	40.6	7.92 ± 0.88	0.48
77/16/7	40.9	8.06 ± 0.75	0.76
49/18/33	40.8	8.03 ± 0.63	1.10

[a] [DPPC] = [PEAA] = 1 mg/mL in 50 mM Tris-HCl, pH 7.4. [b] Polymers are designated by their triad tacticities, given as % I/% H/% S. [c] The temperature scale on the Mc-1 microcalorimeter is uncalibrated. [d] Peak width at half-maximum ΔC_p.

"onion-like" multilamellar vesicles that undergo a sharp order-to-disorder transition at approximately 41 °C.[17] This transition is readily observed by high-sensitivity differential scanning calorimetry.[18] In Figure 2 are shown the melting endotherm for DPPC suspended in 50 mM Tris-HCl buffer at pH 7.4 and similar endotherms for the lipid suspended in the same buffer containing 0.1% of PEAAs of varying tacticity. The calorimetric results are given in quantitative form in Table III.

The transition parameters for the unmodified DPPC sample used in this work are in agreement with the results of previous workers.[19] Hydration of the lipid at pH 7.4 in the presence of PEAA produces a vesicle suspension that melts at essentially the same temperature as the unmodified lipid, but the transition half-width ($\Delta T_{1/2}$) is increased by the polymer. The magnitude of the increase in $\Delta T_{1/2}$ is dependent on the tacticity of the added PEAA. The transition enthalpy (ΔH_m) is unaffected by the polymer under the conditions of these experiments.

In very general terms, one might expect to observe polymer–lipid interactions of three kinds: (i) adsorption of the polymer on the lipid bilayer surface, (ii) insertion of the polymer into the bilayer (by analogy to intrinsic membrane proteins), and (iii) complete disruption of the bilayer, with formation of mixed polymer–lipid micelles or other aggregates. The fact that ΔH_m is unaffected by the polymer suggests that the DPPC/PEAA interaction at pH 7.4 is essentially of the first kind—insertion into or disruption of the bilayer should reduce ΔH_m. Reduction of ΔH_m by reconstitution of intrinsic membrane proteins is well documented,[20] and complete disruption of the bi-

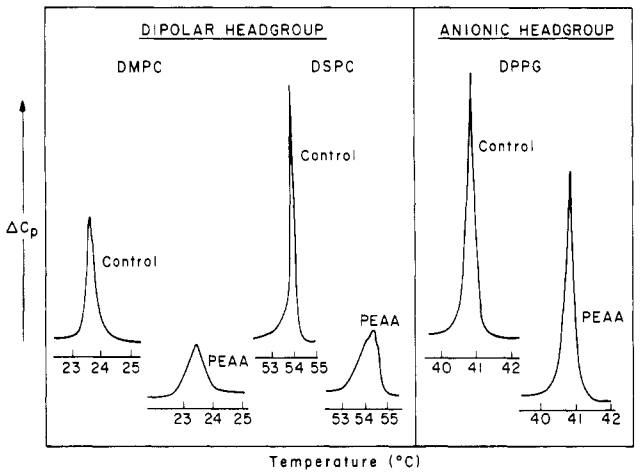


Figure 3. Main phase transition region for phospholipid multilamellar vesicles (1 mg/mL) prepared in 50 mM Tris-HCl buffer (pH 7.4). Controls are lipid alone; endotherms marked "PEAA" were obtained from vesicles hydrated in buffer that contained 1 mg/mL of PEAA 77/16/7.

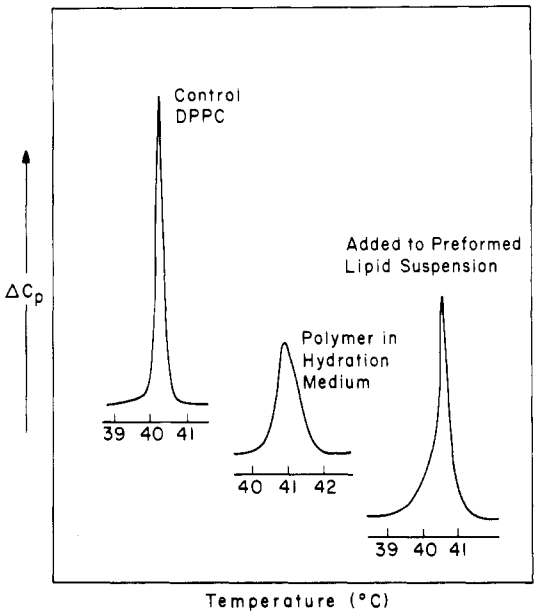


Figure 4. Main phase transition region for DPPC multilamellar vesicles (1 mg/mL) in 50 mM Tris-HCl buffer (pH 7.4). Control is lipid alone; other endotherms were obtained from suspensions containing 1 mg/mL of PEAA 77/16/7, introduced as noted in the figure.

layer should reduce ΔH_m to zero.

Two other sets of experiments are consistent with the hypothesis that PEAA is adsorbed on the DPPC surface at pH 7.4. The first demonstrates the importance of the lipid head group structure. Figure 3 shows a set of experiments in which PEAA 77/16/7 was added to two phosphatidylcholines (DMPC and DSPC) of different hydrocarbon chain lengths and to dipalmitoylphosphatidylglycerol (DPPG), which is negatively charged under the conditions of the experiment. The melting transitions of the phosphatidylcholines are broadened in a manner very similar to that seen for DPPC, while the DPPG endotherm is unchanged. The polymer–lipid interaction thus appears to be insensitive to the lipid acyl chain length (and hence to bilayer thickness) but sensitive to the lipid head group structure. The absence of any effect of PEAA on DPPG at pH 7.4 is no doubt a result of charge repulsion between the negatively charged lipid surface and the ionized carboxylate groups of the polymer. No such repulsion is expected from the dipolar head group of the phosphatidylcholines.

A second set of experiments that is consistent with the adsorption hypothesis concerns the manner in which the polymer is introduced into the lipid suspension. Figure 4 shows the results. When the polymer is present in the hydration medium, a broad endotherm is observed, as in Figure 2. When the polymer is added at room temperature to the *preformed* vesicle suspension, the result is quite different. One observes what may well be a composite melting endotherm—a small broad peak associated with the melting of the outermost bilayer, superimposed on the sharp transition of the inner, "protected" bilayers. The polymer thus appears to be unable to traverse the bilayer and gains access to the inner compartments of the vesicle only if entrapped there during the hydration step.

If one accepts the arguments given above, the next problem is the origin of the tacticity effect shown in Figure 2. One might suspect after an examination of Table II that the important parameter may be molecular weight, rather than tacticity. Although the results are not shown, we have very strong evidence that this is not so. The transition half-widths observed for PEAA-modified DPPC suspensions at pH 7.4 are independent of the molecular weights of the added PEAAs, if tacticity is held constant. An exception to this statement is a marked increase in $\Delta T_{1/2}$ at very low molecular weights ($\eta_{inh} \leq 0.05$ dL/g); the origin of this latter phenomenon may be related to end-group effects but is not understood.

Two general kinds of explanations for the tacticity effect may be considered. The first might be called a "local" effect; perhaps PEAA 49/18/33 broadens the DPPC transition most markedly because heterotactic triads offer a better (or worse) "fit" at the bilayer surface than do isotactic or syndiotactic triads. Alternatively, the tacticity effect may be a "global" one, a result of changes in the acidity, conformation, or solubility of the polymer as the microstructure is varied.

The following experiment provides good evidence that global effects are dominant. Figure 5 shows melting endotherms for DPPC alone and for DPPC treated with PEAA 16/44/40 at pH 7.4 or at pH 7.0. First of all, despite the high concentration of heterotactic triads, PEAA 16/44/40 leaves the melting endotherm essentially unchanged at pH 7.4. Second, the marked broadening of the transition at pH 7.0 demonstrates that small variations in pH—at least within this pH range—influence the polymer–bilayer interaction more strongly than do large variations in polymer microstructure. The effect of pH is explored more fully in the following section.

Interaction of Poly(acrylic acid) Derivatives with Dipalmitoylphosphatidylcholine Vesicles. Effect of pH. Just as PEAA 16/44/40 can be induced to broaden the DPPC phase transition by a decrease in pH from pH 7.4 to pH 7.0, PEAA 77/16/7 appears to be removed from the vesicle membrane by an increase in pH from pH 7.4 to pH 9.0. Figure 6 shows DPPC melting endotherms recorded for lipid suspensions prepared in the presence of PEAA 77/16/7 at several pHs between pH 7.0 and pH 9.0. The high-pH endotherm is identical with that of the control, while increasingly broad transitions are observed at lower pH. A similar effect is noted for a series of poly(methacrylic acids) (PMAAs) of varying tacticity; Figure 7 shows the results for one such sample. The most striking difference between Figures 6 and 7 is the pH at which broadening of the transition is observed: ca. 8 for PEAA vs. ca. 6 for PMAA.

The parameter most convenient as a measure of the polymer–lipid interaction is the transition half-width $\Delta T_{1/2}$.

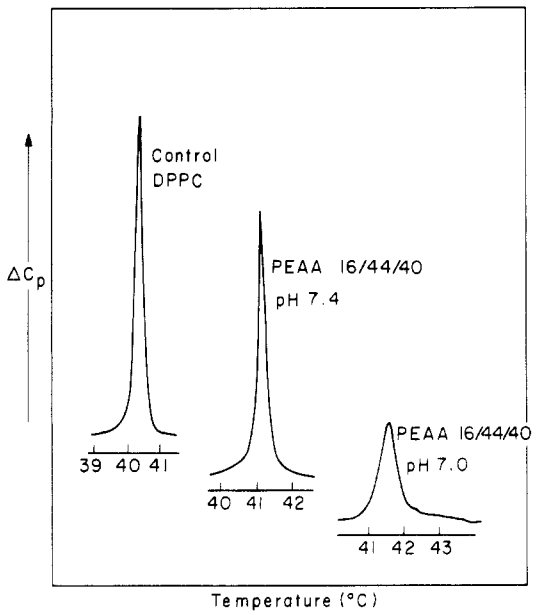


Figure 5. Main phase transition region for DPPC multilamellar vesicles (1 mg/mL) in 50 mM Tris-HCl buffer. Control is lipid alone (pH 7.4); other endotherms were obtained from suspensions hydrated in buffer containing 1 mg/mL of PEAA 16/44/40, at the pH noted in the Figure. Control endotherms are essentially identical between pH 7.0 and pH 9.0.

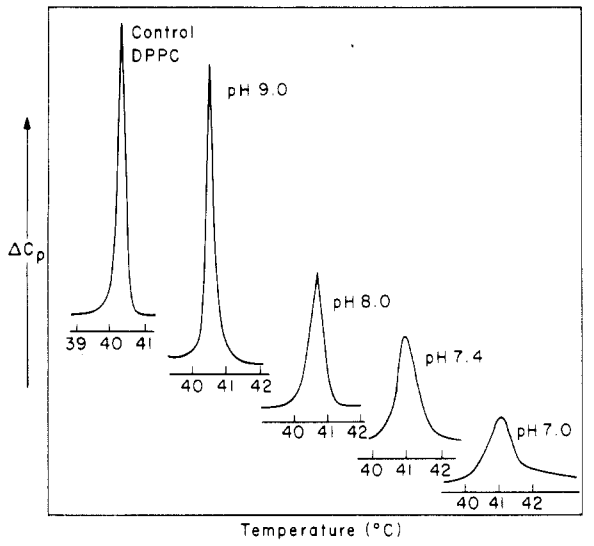


Figure 6. Main phase transition region for DPPC multilamellar vesicles (1 mg/mL) prepared in 50 mM Tris-HCl buffer (pH as indicated) that contained PEAA 77/16/7 (1 mg/mL). Control (pH 7.4) contained no polymer.

Figure 8 shows the dependence of $\Delta T_{1/2}$ on pH for unmodified DPPC vesicles and for vesicles prepared in the presence of two PEAAs, two PMAAs, and a poly(acrylic acid) (PAA). At pH > 8, each of the polymers leaves the DPPC melting endotherm unperturbed, but there is a pH characteristic of each polymer at which $\Delta T_{1/2}$ increases sharply. This "critical pH" depends not only on the chemical structure of the polymer but also on its tacticity. Critical pHs (defined arbitrarily as the midpoint of the rise of the curve in Figure 8) are listed in Table IV.

We have considered a number of molecular interpretations of the results shown in Figure 8. It is not surprising that the polymer–lipid interaction should be most readily observed at low pH, because polymer–water interactions become less favorable as the acidity of the medium increases. For example, the intrinsic viscosity of PEAA 77/16/7 in 50 mM Tris-HCl buffer is 2.67 dL/g at pH 9.0,

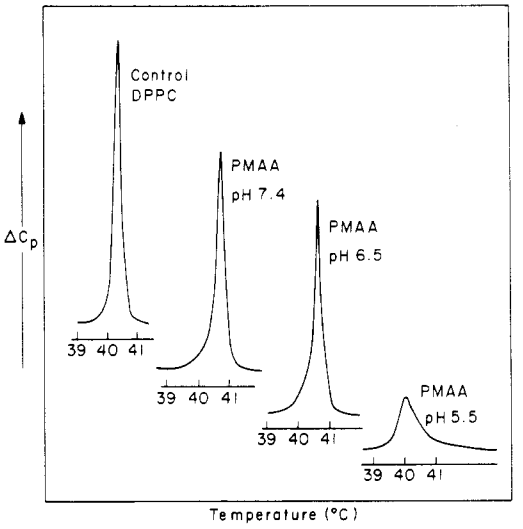


Figure 7. Main phase transition region for DPPC multilamellar vesicles (1 mg/mL) prepared in 5 mM Tris-HCl buffer (pH as indicated) that contained PMAA 59/20/21 (1 mg/mL). Control (pH 7.4) contained no polymer.

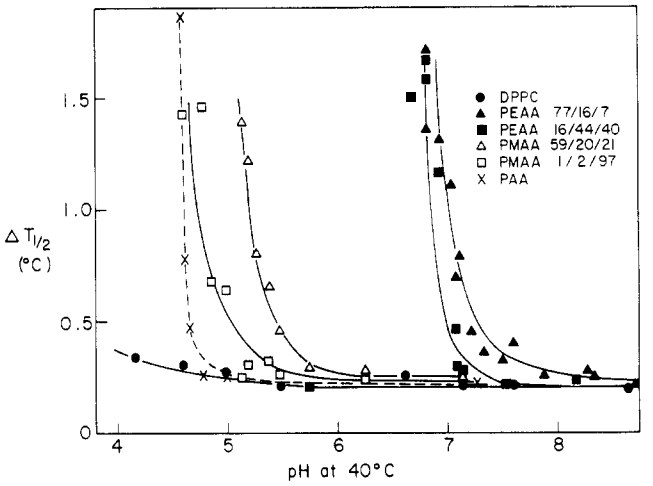


Figure 8. DPPC phase transition half-widths vs. pH at 40 °C: (●) DPPC alone; (▲) in the presence of PEAA 77/16/7; (■) in the presence of PEAA 16/44/40; (△) in the presence of PMAA 59/20/21; (□) in the presence of PMAA 1/2/97; (×) in the presence of PAA. Conditions as in Figure 6.

Table IV
Critical pH for Polymer–DPPC Complexation

polymer	triad tacticity, % I	H	S	crit pH
PEAA	77	16	7	7.2
PEAA	16	44	40	6.8
PMAA	59	20	21	5.3
PMAA	1	2	97	4.9
PAA				4.6

but only 0.80 dL/g in the same buffer at pH 7.0. Since some fraction of the polymer–water interaction must be sacrificed when the polymer binds to the bilayer surface, a decrease in pH should in this way promote binding, regardless of the nature of the polymer–lipid interaction.

Attractive forces between a partially ionized poly(carboxylic acid) and a dipalmitoylphosphatidylcholine bilayer might arise from (i) charge–dipole interactions involving polymer-bound carboxylate anions and the phosphocholine dipole, (ii) hydrophobic interactions involving the hydrocarbon portions of the polymer and the lipid, or (iii) hydrogen bonding involving un-ionized carboxyl groups of the polymer and the phosphodiester head group of the

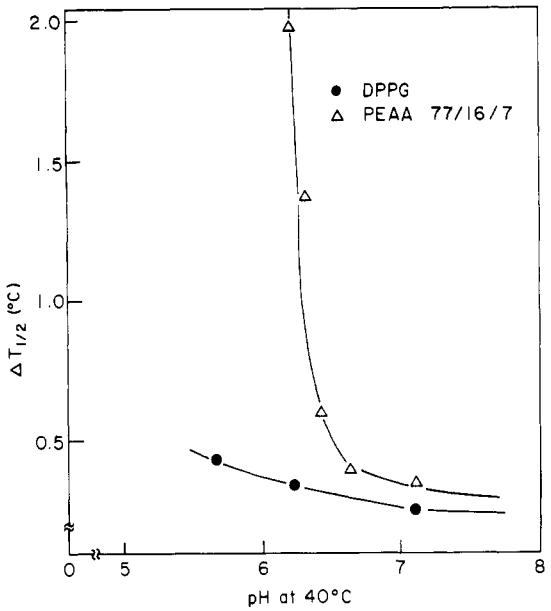


Figure 9. DPPG phase transition half-width vs. pH at 40 °C: (●) DPPG alone (1 mg/mL in 50 mM Tris-HCl); (△) DPPG (1 mg/mL) hydrated in 50 mM Tris-HCl buffer containing PEAA 77/16/7 (1 mg/mL).

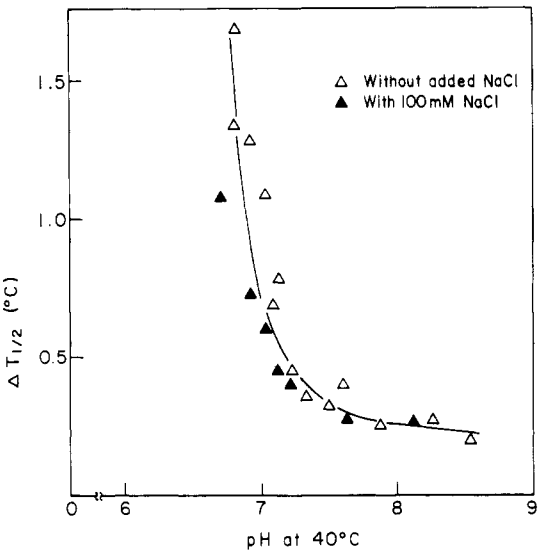


Figure 10. DPPC phase transition half-width vs. pH at 40 °C. Hydration medium was 50 mM Tris-HCl buffer containing PEAA 77/16/7 (1 mg/mL). (△) Without added NaCl; (▲) with 100 mM NaCl.

lipid. Although additional experiments will be required to identify these interactions more directly, we suggest that hydrogen bonding is likely to provide a major driving force for polymer–lipid binding in the systems considered in this paper. Hydrophobic interactions cannot now be discounted with certainty but are probably of restricted importance, because poly(acrylic acid) shows no tendency to bind simple hydrophobic solutes in aqueous solution, even at low pH.[21,22] Charge–dipole interactions can be somewhat more confidently discounted. Although it was shown earlier (Figure 3) that PEAA 77/16/7 causes no modification of the anionic lipid DPPG at pH 7.4, the polymer–lipid charge repulsion is apparently overcome at slightly lower pH, with the result that the DPPG melting endotherm becomes quite broad in polymer solutions of pH < 6.5 (Figure 9). Since the DPPG head group bears only a negative charge, charge–dipole interactions cannot contribute to the polymer–lipid binding in this system.

Thus we suggest that hydrogen bonding provides a major driving force for the binding of poly(carboxylic acids) at the lipid–bilayer surface. Additional observations consistent with this suggestion are the following: (i) the phosphodiester group serves as an H-bond acceptor in crystals of lipid hydrates,[23] (ii) poly(carboxylic acids) form strong interpolymer complexes with H-bond acceptor polymers such as poly(ethylene oxide) (the pH dependence of interpolymer complex formation is similar to that observed in this work),[24] and (iii) the interaction of PEAA 77/16/7 with DPPC is not effectively screened by added electrolyte (Figure 10 shows plots of $\Delta T_{1/2}$ vs. pH in 50 mM Tris-HCl buffer with and without 100 mM NaCl; the results are nearly identical). Hydrogen-bonding interactions at the lipid surface would be expected to be insensitive to added electrolyte.

This hypothesis accounts for the observed pH dependence of binding as a result of a cooperative hydrogen bonding of un-ionized carboxyl groups with the phosphodiester functions on the bilayer surface. It may be that losses of translational and configurational entropy that accompany binding must be overcome by the formation of a critical number of hydrogen bonds per polymer chain; at low extents of protonation, this situation canot be realized. Why such an interaction should cause a loss in cooperativity of the lipid melting transition is not clear, but perhaps a mismatch of optimal geometries for lipid packing and polymer–lipid hydrogen bonding disorders the bilayer structure and disrupts the cooperative melting process. Spectroscopic experiments now in progress are designed to provide direct evidence for or against the existence of polymer–lipid hydrogen bonds in these systems.

Conclusions

The structural properties of phospholipid vesicle membranes are modified by complexation with poly(carboxylic acids). Complexation is strongly dependent on the pH of the medium and may be controlled by variation in polymer chemical structure and tacticity. The latter variables appear to operate on the complexation process through their effects on the pK_a's of the polyacids. Complexation is absent at high pH, becomes apparent through broadening of the lipid phase transition as pH decreases, and finally (in PEAA solutions) causes the disappearance of large multilamellar lipid vesicles. A primary driving force for polymer–lipid complexation is suggested to be the formation of hydrogen bonds between un-ionized polymer-bound carboxyl groups and the phosphodiester functions of the lipid surface. Further characterization of these systems and their application in process requiring pH-dependent delivery of vesicle contents are under way.

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Comparison of Experimental and Theoretical Persistence Length of Some Polyelectrolytes at Various Ionic Strengths

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ABSTRACT: The literature values of the intrinsic viscosity-molecular weight dependence of some polyelectrolytes have been analyzed on the basis of Yamakawa's theory in order to derive the persistence length q with a computer fitting procedure. Sodium polyacrylate, poly(styrenesulfonate), poly[((acrylamido)-methyl)propanesulfonate], (carboxymethyl)cellulose, and the sodium salt of an isobutyl vinyl ether-maleic anhydride copolymer have been investigated in the 1.0-0.005 M ionic strength range. The persistence lengths l_p, determined by extrapolation to infinite ionic strength with a linear q vs. $I^{-1/2}$ plot, agreed with those of the corresponding uncharged polymers. In addition, the q values of the vinylic polyelectrolytes appeared to extrapolate toward a similar value, about 500 Å, at infinite dilution, indicating a high degree of rigidity. The observed $I^{-1/2}$ dependence of the electrostatic contribution to the persistence length l_e differed from the I^{-1} dependence predicted in the Odijk-Skolnick-Fixman theory and agreed, at least semiquantitatively, with that calculated from the recent treatments of Le Bret and Fixman. The influence of the ionization degree on the persistence length of sodium polyacrylate was also studied.

Introduction

During the past 5 years, several attempts have been made to theoretically express the persistence length of polyelectrolytes as a function of ionic strength in order to obtain an experimental value of this parameter from the molecular weight dependences of hydrodynamic data (intrinsic viscosity, radius of gyration). Since the persistence length, equal to half the Kuhn statistical segment, is a measure of how far a polymeric chain persists in a given direction, it really defines the chain rigidity. In the particular case of polyelectrolytes, the persistence length is a sum of two contributions: a bare persistence length l_p due to the rigidity of the chain backbone and an electrostatic persistence lenth l_e arising from the repulsion between neighboring ionic sites, the latter being markedly dependent upon the concentration of added salt. Theoretical estimation of l_e has been achieved by Odijk,[1-4] Skolnick and Fixman,[5] and recently by Fixman[6] and Le Bret.[7,8]

Presently, the most commonly used relationship for the determination of the persistence length from viscosity results is that established for the case of wormlike chains

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